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Vectorial Targeting of an Endogenous Apical Membrane Sialoglycoprotein and Uvomorulin in MDCK Cells

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Abstract. We studied the cell-surface delivery pathways of newly synthesized membrane glycoproteins in MDCK cells and for this purpose we characterized an endogenous apical integral membrane glycoprotein. By combining a pulse-chase protocol with domain-selective cell-surface biotinylation, immune precipitation, and streptavidin-agarose precipitation (Le Bivic et al. 1989. *Proc. Natl. Acad. Sci. USA.* 86:9313–9317), we followed the appearance at the cell surface of a major apical sialoglycoprotein, gp14, a basolateral protein, ovomorulin, and a transcytosing protein, the polyimmunoglobulin receptor (pIg-R). We determined that both gp14 and ovomorulin appeared to be delivered directly to their respective surface, with mistargeting levels of 8 and 2%, respectively. Using the same technique, the pIg-R was first detected on the basolateral domain and then on the apical do-

main, to be finally released into the apical medium, as described (Mostov, K. E., and D. L. Deitcher. 1986. *Cell.* 46:613–621). To directly determine whether the gp14 pool present on the basolateral surface was a precursor of the apical gp14, we compared it with the equivalent pIg-R pool, by labeling with sulfo-NHS-SS-biotin, a cleavable, tight junction-impermeable probe, and by following the fraction of this probe that became resistant to basal glutathione and accessible to apical glutathione during incubation at 37°C. We found that, contrary to pIg-R, basolateral gp14 was poorly endocytosed and was not transcytosed to the apical side. These results demonstrate that an endogenous apical integral membrane glycoprotein of Madin-Darby canine kidney cells is sorted intracellularly and is vectorially targeted to the apical surface.

MDCK cells have been extensively used to study the biogenesis of epithelial cell polarity (6, 18, 26a, 31). Studies on the sorting of plasma membrane proteins were initially carried out with MDCK cells infected with enveloped RNA viruses, such as influenza and vesicular stomatitis virus, which bud, respectively, from the apical or the basolateral domains of the plasma membrane (27). Polarized viral budding is preceded by segregation of the main envelope glycoproteins, i.e., influenza HA and vesicular stomatitis virus G protein into the budding domain (26). Furthermore, when these proteins were expressed in MDCK cells from transfected cDNAs they displayed a similar polarized distribution to that observed in infected cells (11, 28). Taking advantage of these powerful viral tools, several studies established that, in MDCK cells, the polarized distribution of viral envelope glycoproteins is determined by intracellular sorting at the level of the *trans*-Golgi network and vectorial delivery to the respective surface (19, 20, 23, 24).

Because of their lower biosynthetic rates, and the corresponding need for higher sensitivity methods, studies on the targeting of endogenous MDCK glycoproteins have lagged behind those on viral glycoproteins. Only one paper has appeared, by Caplan et al. (1986), reporting the direct delivery of a basolateral membrane pump, the Na⁺,K⁺ ATPase, to

the basolateral surface [7]. The need for additional studies on the pathways of apical glycoproteins in MDCK cells is stressed by observations in native intestinal and liver epithelia, which suggest an indirect pathway for apical membrane proteins: delivery to the basolateral domain followed by relocation to the apical domain (2, 13, 17).

Largely, most studies available in both MDCK and native epithelial cells base their conclusion on vectorial versus transcytotic targeting on the “kinetics” of the appearance of the proteins at both surfaces, but not on the direct analysis of the fate of the protein pool present in the “incorrect” surface. In this report, we apply to MDCK cells three experimental strategies that we recently introduced to study the targeting of apical and basolateral integral membrane glycoproteins in a human intestinal cell line (15). The first one is analogous to the approach used by Lisanti et al. (16) (pulse-chase and domain-selective biotinylation), except that the proteins were precipitated with specific antibodies (instead of lectins) to highly increase the sensitivity of the method. The second one involves a procedure to directly observe the fate of the basolateral pool of apical antigens; this method involves labeling with a cleavable biotin analog to measure their endocytotic and transcytotic rates (15). Finally, the pathways of the endogenous antigens are compared with that

of exogenous polyimmunoglobulin receptor (pIg-R)¹, which includes a basolateral stage in its complex route to the apical surface and therefore constitutes an ideal positive control (21). Our results conclusively demonstrate direct targeting of an apical integral membrane glycoprotein and a basolateral cell adhesion molecule (uvomorulin) to their respective surface in MDCK cells.

Materials and Methods

Reagents

Cell culture reagents were purchased from Gibco Laboratories (Grand Island, NY). Affinity-purified antibodies, rabbit anti-mouse IgG, rhodamine-conjugated goat anti-mouse IgG and goat anti-guinea pig IgG were purchased from Cappel Laboratories (Westchester, PA). Protein A-Sepharose was from Pharmacia (Uppsala, Sweden), sulfo-N-hydroxy-succinimido-biotin (s-NHS-biotin), sulfosuccinimidyl 2-(biotinamido) ethyl 1,3-dithiopropionate (s-NHS-SS-biotin), and streptavidin-agarose beads were from Pierce Chemical Co. (Rockford, IL). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Cells, Antibodies, and Cell Culture

MDCK type II were grown in DME supplemented with 10% horse serum. MDCK cells expressing the cDNA for the pIg-R have been described previously (21) and were grown in DME supplemented with 5% FBS. Guinea pig antiserum for the pIg-R has been described elsewhere (4). mAb against uvomorulin was a generous gift from Dr. B. Gumbiner (University of California at San Francisco) (12). Rabbit polyclonal antibodies against gp114 were obtained by purification of this protein from apical membrane preparations (29) by wheat germ agglutinin (WGA)-Sepharose affinity chromatography (22) and separation on SDS-PAGE (14). After electrophoretic transfer to nitrocellulose paper (33), the band was visualized by Ponceau red, cut out, and injected into New Zealand rabbits for immunization (10). For experiments, cells were grown on Transwells (Costar Data Packaging Corp., Cambridge, MA) and used after 7 d.

Biotinylation

Biotinylation of monolayers on Transwells with s-NHS-biotin (30) was carried out twice in a row for 20 min at 4°C with 0.5 ml for the apical chamber and 1.5 ml for the basolateral chamber. Free biotin was blocked with 50 mM NH₄Cl in PBS containing 1 mM MgCl₂ and 0.1 mM CaCl₂. Biotinylation with s-NHS-SS-biotin was performed as for s-NHS-biotin. After biotinylation, reduction of surface s-NHS-SS-biotin was performed with 50 mM glutathione for 30 min (15) in 90 mM NaCl, 1 mM MgCl₂, 0.1 mM CaCl₂, 60 mM NaOH, and 10% FBS.

Pulse-Chase Experiments

Cells grown on filters were incubated for 30 min in DME without methionine/cysteine, and pulsed for 20 min in the same medium containing 0.8 mCi/ml ³⁵S label (ICN K&K Laboratories Inc., Plainview, NY) and 0.4 mCi/ml ³⁵S cysteine (NEN, Chadds Ford, PA) as described (21). Cells were washed once with DME, chased in DME containing 10× cysteine/methionine, and stored at 4°C in NaCO₃H-free DME, 20 mM Hepes, and 0.2% BSA before biotinylation.

Immunoprecipitation and Streptavidin Precipitation

After biotinylation, filters were excised and cells were solubilized in 1 ml of lysis buffer: 150 mM NaCl, 20 mM Tris pH 8.0, 5 mM EDTA, 1% Triton X-100, 0.2% BSA, and protease inhibitors for 1 h under agitation. Extracts were precleared by addition of 100 μl of a *Staphylococcus aureus* slurry (fixed bacteria, 50% vol/vol, prewashed three times) (Pansorbin; Calbiochem-Behring Corp., San Diego, CA) for 20 min and centrifuged at 15,000 g for 10 min. Supernatants were incubated for 12 h with protein A-Sepharose (10 mg/ml) precoated with rabbit anti-mouse Ig plus mAbs (diluted 1/100 for ascites) or rabbit polyclonal antibodies (1/250) for gp114 or guinea pig

polyclonal antibodies (1/4,000) for pIg-R. After incubation, the beads were washed as described (15). To recover the immunoprecipitated biotinylated antigens, the beads were boiled with 10 μl of 10% SDS for 5 min, diluted with lysis buffer (500 μl/tube), and centrifuged (1 min at 15,000 g). Supernatants were incubated overnight with streptavidin-agarose beads (50 μl, 50% slurry). Finally, the beads were washed (15) and boiled in gel sample buffer and analyzed by SDS-PAGE 6/16% (14). Dried gels were processed for fluorography as described (8) using preflashed films. Densitometric analysis was performed under conditions where linearity was best preserved using a scanning densitometer (model GS 300; Hoefer Scientific Instruments, San Francisco, CA); at least two independent experiments were performed. Alternatively, immunoprecipitated antigens from biotinylated cells were directly analyzed by SDS-PAGE under reducing conditions for s-NHS-biotin or nonreducing conditions for s-NHS-SS-biotin, and blotted with ¹²⁵I-streptavidin on nitrocellulose (30).

Frozen Sections

0.5-μm frozen sections of MDCK cells on collagen (32) were processed for immunofluorescence as described (15).

Results

Characterization of the Surface Distribution of Two Endogenous and One Exogenous Integral Membrane Glycoproteins of MDCK Cells

In a previous study (29), two major sialoglycoproteins (approximate molecular mass 100 and 200 kD) were detected in isolated apical membrane fractions of MDCK cells by ¹²⁵I-WGA blotting. The lowest molecular weight band was unextractable with carbonate buffer (29) and partitioned with the detergent phase of Triton X-114 (16), which indicated that it is an integral membrane protein. This protein was purified from isolated apical membranes of MDCK cells by affinity chromatography on a WGA-Sepharose column (22) and used to prepare polyclonal antibodies. Using this antibody on semi-thin frozen sections of MDCK cells the antigen was localized at the apical surface (Fig. 1, *a* and *b*). In contrast, an mAb against the cell adhesion molecule uvomorulin labeled mainly the lateral membrane of the cells (Fig. 1, *c* and *d*). Immunoprecipitation from cells labeled with s-NHS-biotin revealed only a 114-kD protein (gp114); in filter-grown monolayers, 95% of gp114 was labeled from the apical side (Fig. 2), confirming its apical localization. Under identical conditions, uvomorulin was preferentially labeled (96%) from the basolateral side (Fig. 2). Rabbit pIg-R, permanently expressed in MDCK cells by transfection of its cDNA (21) appeared also basolaterally polarized (94%) with the biotinylation procedure (Fig. 2).

Surface Delivery of gp114, Uvomorulin, and pIg-R

The biosynthesis of gp114 was followed by pulse-chase with ³⁵S methionine/³⁵S cysteine and immunoprecipitation. After a 20-min pulse, a precursor 85-kD form was rapidly converted into the mature 114-kD form within the first 45 min of chase (Fig. 3). The surface appearance of gp114 and uvomorulin on confluent monolayers of MDCK cells grown on Transwells was studied by metabolic labeling with a ³⁵S-methionine/³⁵S-cysteine pulse (20 min), followed by chase in a medium containing an excess of cold methionine and cysteine. At different times of chase, the cells were labeled either on their apical or their basolateral side with s-NHS-biotin and the antigens were immunoprecipitated, released from the beads, and precipitated with streptavidin coupled

1. **Abbreviations used in this paper:** pIg-R, polyimmunoglobulin receptor; WGA, wheat germ agglutinin.

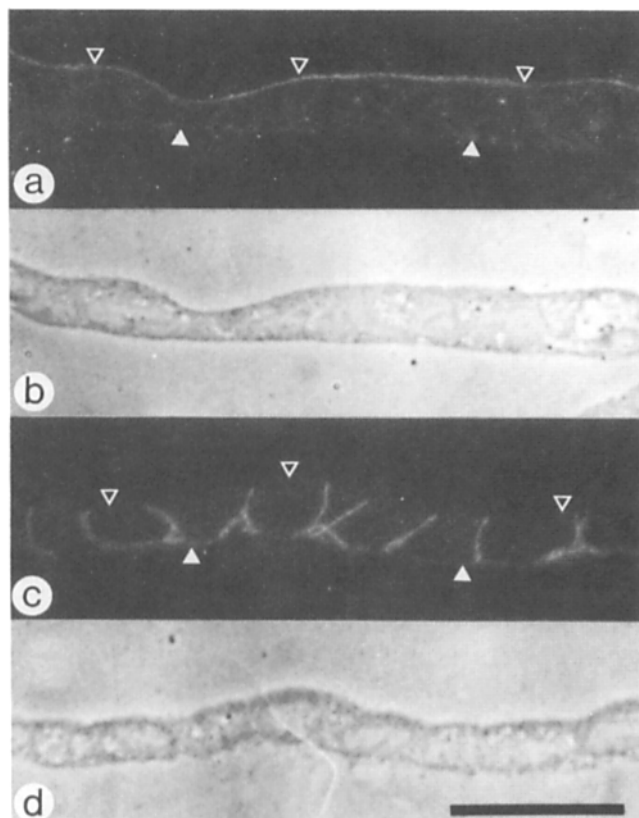


Figure 1. Indirect immunofluorescence localization of gp114 (a and b) and uvomorulin (c and d) on semi-thin frozen sections of MDCK cells grown on collagen. Gp 114 is localized mainly on the apical side of the cells (empty arrowheads) while uvomorulin is present only on the basolateral membrane (white arrowheads). Bar, 10 μ m.

to agarose beads (15). Gp114 arrived at the apical surface with a half time of 45 min, while uvomorulin made its appearance on the basolateral membrane with a half time of 35 min (Figs. 4 and 5). The time between the disappearance of the precursor form and the surface appearance of the mature form was 15 min for gp114 and 20 min for uvomorulin (Fig. 5). Both antigens seemed to be delivered directly to their respective surface with 2% missorting for uvomorulin and 8% missorting for gp114 (Fig. 5). At any time of the chase

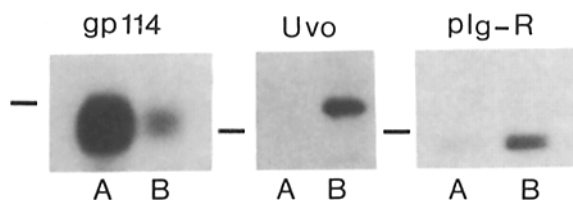


Figure 2. Immunoprecipitation of gp114, uvomorulin (Uvo), and pIg-R after surface labeling of the apical (A) or the basolateral (B) sides of MDCK cell monolayers. The biotinylated proteins were revealed after SDS/6–16% PAGE and transfer to nitrocellulose by 125 I-streptavidin blotting. Molecular weight marker is 116 kD. Gp114 was mainly detected in apically labeled cells while uvomorulin and pIg-R were mainly detected in basolaterally labeled cells. The autoradiogram presented here for gp114 was overexposed to show the basolateral pool of gp114 at the steady state.

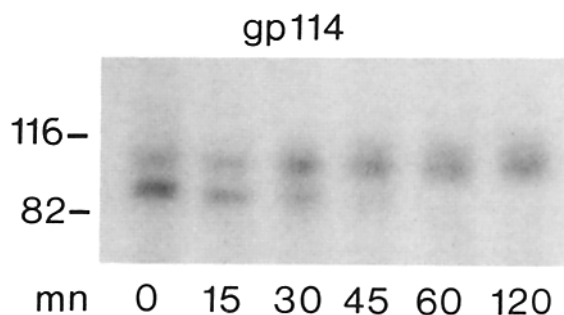


Figure 3. Biosynthesis of gp114 in MDCK cells. Cells were pulsed with 35 S methionine and 35 S cysteine for 20 min and chased for the time indicated. Gp114 was immunoprecipitated, analyzed by SDS/6–12% PAGE, and processed for fluorography. An 85-kD precursor form progressively matures into a 114-kD form. Molecular mass markers are in kD.

more gp114 was present on the apical than on the basolateral surface of the cells (Fig. 5) and no peak of gp114 could be detected on the basolateral membrane. Furthermore, the level of gp114 on the basolateral surface appeared to be steady even after 280 min of chase.

Previous work with native liver and intestinal cells has suggested that certain apical proteins may appear transiently in the basolateral surface before final transfer to the apical surface (2, 13, 17). To rule out the possibility that a similar transient appearance of gp114 in the basolateral surface of MDCK cells was missed by our domain-selective biotinylation procedure, we carried out two types of experiments. First, we followed the cell-surface appearance of a protein for which this pathway has been very well documented, namely, the pIg-R transfected into MDCK cells (21). As described above for gp114 and uvomorulin, MDCK monolayers were pulsed (10 min) with 35 S-cysteine, chased for various times, and subjected to domain-selective biotinylation, immunoprecipitation, and streptavidin-agarose precipitation. The pIg-R was first detected on the basolateral surface with a half time of 30 min, then on the apical membrane with a half time of 65 min, and was finally secreted into the apical medium with a half time of 135 min (Fig. 5). These results confirm the basolateral to apical transcytosis described for the pIg-R in MDCK cells (4, 21) and show that the procedure we used is sensitive enough to detect the transient appearance of this receptor in the basolateral membrane. However, a very fast transit of gp114 might have gone undetected. Therefore, as a second approach, we decided to study directly the fate of the basolateral pool of gp114.

Fate of the Basolateral Pool of gp114 and pIg-R

For this purpose, we used our recent modification (15) of the technique described by Bretscher and Lutter (5). Confluent monolayers of MDCK cells grown on filters were labeled at 4°C (time 0) from the basolateral side with a cleavable analogue of s-NHS-biotin, s-NHS-SS-biotin. After labeling, the cells were warmed up for different times to allow endocytosis and the biotin remaining at the cell surface was stripped by reduction with 50 mM glutathione (15). The cells were then extracted and the biotinylated antigens were immunoprecipitated, analyzed by SDS-PAGE under nonreducing conditions, and detected by 125 I-streptavidin blotting. Endocy-

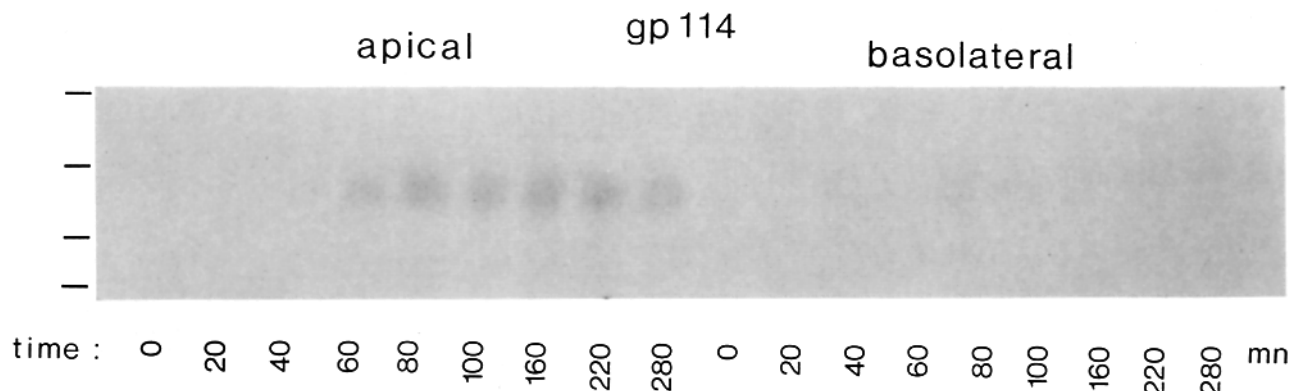


Figure 4. Appearance at the cell surface of gp114 in MDCK cells. Cells were pulsed with ^{35}S methionine and ^{35}S cysteine for 20 min and chased for the times indicated. Newly synthesized gp114 was detected at the cell surface as described in Materials and Methods. Immunoprecipitated gp114 was analyzed by SDS/6–16% PAGE and fluorography. Molecular mass standards are from top to bottom 180, 116, 82, and 58 kD.

tosis of a given antigen was measured as the increase in the amount of biotinylated antigen that became resistant to glutathione reduction.

To determine the efficiency of the technique, we studied the fate of pIg-R in transfected MDCK cells. All the label incorporated from the basolateral side into pIg-R (Fig. 6, lane *a*) could be removed by the addition of glutathione at time 0 to the basolateral side (lane *d*) but not to the apical side (lane *g*) of the monolayer. On the other hand, incubation at 37°C resulted in fast protection of the biotinylated pIg-R to reduction by glutathione from the basolateral side. Almost all pIg-R was endocytosed by 30 min (lanes *e* and *f*) and became sensitive to apical reduction by about the same time (lane *h* and *i*), consistent with the reported transcytotic pathway of this receptor (4, 21). The cleaved form of pIg-R was detected in the apical medium somewhat later, by 120 min (lanes *j* and *k*). Thus, biotinylation of pIg-R did not prevent its normal transcytosis to the apical side or its release by proteolytic cleavage into the apical medium. In combination with glutathione reduction, this method allowed us to follow the fate of the basolateral pool of the receptor.

We applied the same technique to follow the fate of the basolateral pool of gp114 by labeling the cells with s-NHS-SS-biotin at 0°C followed by incubation at 37°C. Even after 2 h at 37°C, gp114 labeled at time 0 on the basolateral surface was still sensitive to basolateral reduction (Fig. 7, lanes *g-i*) and insensitive to apical reduction (lanes *d-f*), indicating that very little transcytosis had occurred. Furthermore, when the rate of endocytosis of basolateral gp114 was compared with that of apical gp114, they were found to be very slow in both cases (Fig. 8). Taken together, these results show clearly that the gp114 molecules present on the basolateral surface do not behave as a transient precursor pool to apical gp114 but, rather, as a stable missorted population.

Discussion

As part of our effort to elucidate the mechanisms involved in the establishment and maintenance of epithelial cell polarity, we characterized the biogenetic pathway of an integral membrane sialoglycoprotein of the apical surface of MDCK cells and compared it with the corresponding pathways of a basolateral protein, uvomorulin, and a transcytosing recep-

tor, pIg-R. Gp114 was shown to be an apical component of MDCK cells by several criteria: (*a*) it is one of the major WGA-binding sialoglycoproteins detected in an apical membrane preparation (29), from which we describe its purification in this report; (*b*) it was localized to the apical pole by frozen sections; and (*c*) by domain-selective cell surface labeling with s-NHS-biotin. Gp114 is an integral membrane glycoprotein because it partitions with Triton X-114 (16) after phase separation (3) and is not extractable by high pH (29).

Gp114 was used as a model to study the surface delivery of apical proteins in MDCK cells. Using a combination of pulse-chase with ^{35}S -methionine/ ^{35}S -cysteine, domain-selective surface biotinylation at different times of chase followed by immune- and streptavidin-agarose precipitation, gp114 appeared to be directly targeted to the apical membrane. Several results strongly suggested this pathway. At any time of the chase, we detected more gp114 on the apical than on the basolateral domain, and the gp114 present on the basolateral membrane remained stable for at least 280 min with no observable peak, compatible with its being a missorted population. Furthermore, the times between Golgi processing of precursor gp114, as detected by shift to an Endo H-resistant, higher molecular weight form, and the basolateral or apical surface appearance of the processed form were very short and similar (~15 min). By comparison, the predominantly lateral cell adhesion molecule uvomorulin was directly targeted to the basolateral membrane, with only 2% being missorted to the apical side, as shown for Na^+, K^+ -ATPase (7) using domain-selective binding of photoactivatable ouabain and immunoprecipitation with antiouabain antibodies.

That the surface-delivery assay described in this paper is sensitive enough to detect transient appearance of a protein in the basolateral membrane was shown by studies with MDCK cells permanently expressing pIg-R (21). Previous studies using antibody binding have shown that this receptor is initially targeted to the basolateral membrane of the MDCK cells and is then transcytosed to the apical membrane and shed into the apical medium by proteolytic cleavage (4, 21). Using the same protocol as for gp114 we showed that we could first detect the appearance of newly synthesized pIg-R on the basolateral surface followed by its arrival

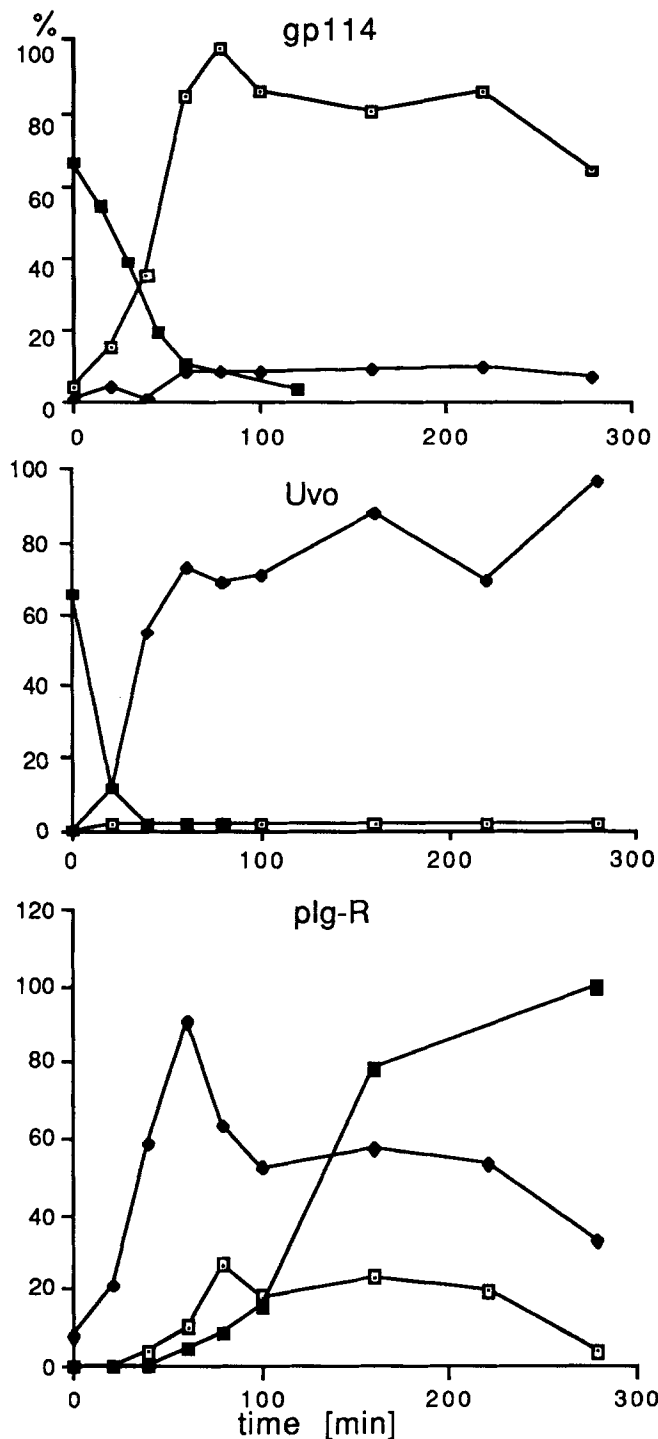


Figure 5. Appearance at the cell surface of newly synthesized gp114, uvomorulin (*Uvo*), and pIg-R. Cells were pulsed for 20 min (gp114 and uvomorulin) or 10 min (pIg-R) and chased for the time indicated. Fluorograms were scanned as described in Materials and Methods and the results were expressed as a percentage of the amount at the time of maximal expression at the cell surface. The secretory component was expressed independently as a percentage of the maximal amount recovered in the apical medium at $t = 280$ min. Precursor (■), apical (□), basolateral (◆), and secreted (●) forms.

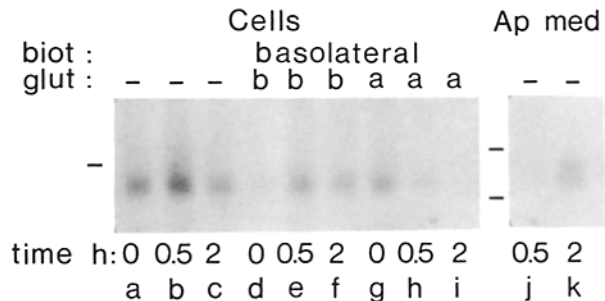


Figure 6. Transcytosis of pIg-R in transfected MDCK cells. Endocytosis and transcytosis of basolateral pIg-R was followed using a procedure derived from Bretcher and Lutter (5). Confluent monolayers were labeled with s-NHS-SS-biotin (*biot*) from the basolateral side at 4°C and reduced with glutathione (*glut*) from the apical (*a*) or the basolateral (*b*) side, immediately or after incubation at 37°C for 0, 0.5, or 2 h. The pIg-R was immunoprecipitated, analyzed by SDS/6–12% PAGE under nonreducing conditions, and revealed by 125 I-streptavidin blotting. The secretory component released into the apical medium was also analyzed (*Ap med*). Molecular mass standards are 116 kD for the left panel and 82 and 58 kD for the right panel. The pIg-R was endocytosed (lanes *d–f*), transcytosed (lanes *g–i*), and secreted into the apical medium (lanes *j* and *k*).

at the apical surface and finally its release into the apical medium. We believe that domain-selective labeling with s-NHS-biotin has several advantages over surface immunoprecipitation. The accessibility of the basolateral domain should be more facile for biotin (mol wt ~400) than for antibodies (mol wt ~150,000) or even Fab fragments (mol wt ~50,000). This may be one of the reasons why we find a higher percent of basolateral pIg-R, 95%, rather than the 50%/20% level reported by Breitfeld et al. (4) using antibody (Fab) or ligand (dimeric IgA) binding; another reason for the low apical level of pIg-R may be recycling to an apical endocytic compartment (4). In addition, biotin labeling has been shown to be restricted to the cell surface (30), while surface immunoprecipitation can be contaminated by intracellular antigen during cell lysis, resulting in an overestimation of the percent of surface protein.

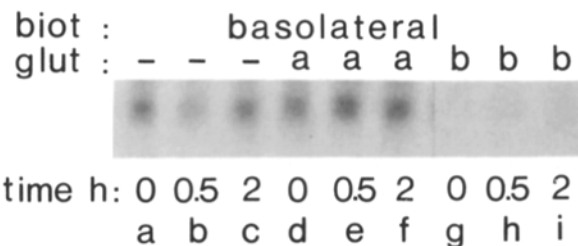


Figure 7. Fate of the basolateral pool of gp 114. Endocytosis and transcytosis of basolateral gp114 was studied by labeling the cells with s-NHS-SS-biotin (*biot*) from the basolateral side at 4°C and by reducing with glutathione (*glut*) from the apical (*a*) or the basolateral (*b*) side, immediately or after incubation at 37°C for 0, 0.5, or 2 h. Gp114 was immunoprecipitated, analyzed by SDS/6–12% PAGE under nonreducing conditions, and revealed by 125 I-streptavidin blotting. Gp114 was poorly endocytosed (lanes *g–i*) and transcytosed (compare lanes *a–c* with *d–f*).

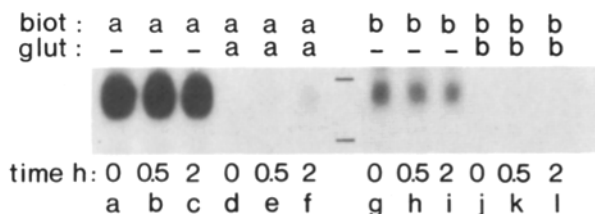


Figure 8. Endocytosis of gp14 from the apical or the basolateral side. Cells were labeled with s-NHS-SS-biotin (*biot*) from the apical (*a*) or basolateral (*b*) sides at 4°C and reduced with glutathione (*glut*) from the apical (*a*) or the basolateral (*b*) side, immediately or after incubation at 37°C for 0, 0.5, or 2 h. Gp14 was immunoprecipitated and analyzed by SDS/6–12% PAGE under nonreducing conditions and revealed by ¹²⁵I-streptavidin blotting. Very little endocytosis was observed even after 2 h from both apical and basolateral sides (compare lane *c* with *f* and lane *i* with *j*). Molecular mass standards are 116 and 82 kD.

Although the results with pIg-R clearly show that we were able to detect a flux of protein in the basolateral membrane, they did not rule out completely a fast transit of gp14 through the basolateral domain. To directly study the fate of the basolateral pool of gp14 we used a new assay (15), derived from a procedure by Bretscher and Lutter (5). This assay employs a cleavable analog of s-NHS-biotin that is membrane impermeable and does not significantly cross the tight junctions. Using this analog, s-NHS-SS-biotin, we found the same polarity ratio for gp14 as found with s-NHS-biotin (compare Fig. 2 and 8). To remove the biotin present at the cell surface we used glutathione (15), that is membrane and tight junction impermeable, at least within the times used (see Fig. 8). By combining basolateral biotinylation of MDCK cells expressing the pIg-R and domain selective reduction with glutathione, we showed that, when the cells were incubated at 37°C, the pIg-R was endocytosed and transcytosed to be released into the apical medium. However, using the same protocol, the basolateral pool of gp14 was shown to be endocytosed very slowly and not significantly transcytosed. This was not likely due to inactivation by biotin labeling since for the pIg-R the normal pathway was not affected. These results indicate that gp14 has a long residence time in the basolateral membrane, where it behaves as a stable pool of newly synthesized protein. Our results are consistent with the hypothesis of intracellular sorting and vectorial delivery of plasma membrane proteins in MDCK cells (16, 19, 20, 23, 24).

It is of great interest to apply the methods used here to the study of the biogenetic pathways of apical proteins in intestinal cell lines. It has been proposed that in the enterocyte two of the major apical hydrolases, sucrase-isomaltase and aminopeptidase N, are sorted at the level of the basolateral domain (13, 17). Recently, we found that one apical and one basolateral protein are sorted intracellularly in an intestinal cell line, SK-CO-15 (15). However, using an identical approach in CaCo-2 cells, newly synthesized aminopeptidase was detected transiently on the basolateral surface while basolateral alkaline phosphatase seemed to behave as a stable mis-sorted pool (like gp14 in MDCK cells) (Le Bivic, A., A. Quaroni, B. Nichols, and E. Rodriguez-Boulant, manuscript submitted for publication). The study of other apical proteins will be necessary to define the prevalence of direct

versus indirect pathways in intestinal cells. It will be particularly informative to study proteins that, endogenously or by transfection, are expressed in both liver and a cultured epithelial line. In this regard, it is interesting to note that gp14 shares many common features with two apical proteins in the liver, the gp10 and HA4 (1, 9). They all bind WGA and are integral membrane proteins heavily glycosylated with core proteins having similar molecular weights (1, 9). Comparing these proteins would be of interest, considering that an indirect pathway was proposed for HA4 (2) in liver.

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Note Added in Proof. While this paper was in press, a report by K. Matter, M. Brauchbar, K. Bucher, and H.-P. Hauri (*Cell*, 1990, 60:429–437) showed that several apical hydrolases follow both vectorial and transcytotic routes to the apical surface of intestinal (CaCo-2) cells. In their study, Matter et al. employed various types of targeting assays, including biotin targeting assays similar to those that we recently published (15, 16) and that we use in this report.

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